In the Specification

At page 1, line 2 of the specification, please insert the following paragraph:

Cross-Reference to Related Applications

This application is a national stage filing of PCT/US99/28033, filed November 24, 1999, which claims the benefit of the filing date of U.S. application Serial No. 60/109,687, filed November 24, 1998.

Please amend the paragraph at page 5, line 7 of the specification as follows:

Figure 5A shows the results of G418 selection ~~(shaded area)~~ of outgrowth endothelial cells stably transfected with a vector encoding eGFP.

Figure 5B shows the morphology (left) and fluorescence (right) of PLE9 transduced cells at day 53.

In the Claims

1. (Previously presented) A process for expanding a population of endothelial cells obtained from peripheral blood comprising culturing, in contact with a collagen I-coated surface, buffy coat cells which are obtained from peripheral mammalian blood, in the presence of a cell culture medium containing an effective amount of vascular endothelial growth factor (VEGF), and which medium is free of bovine brain extract, so as to expand the population of endothelial cells in said buffy coat cells.

2. (Original) The process of claim 1 wherein the blood is human blood.

3. (Original) The process of claim 1 wherein said cell culture medium comprises heparin, dextran sulfate or mixtures thereof.

4. (Original) The process of claim 1 wherein the buffy coat cells are obtained by washing cells from a buffy coat layer obtained from human blood in cell culture medium comprising 20% human male serum.

5. (Original) The process of claim 1 wherein the cell culture medium comprises human basic fibroblast growth factor.

6. (Original) The process of claim 1 or 5 wherein the cell culture medium comprises insulin-like growth factor.

7. (Original) The process of claim 1 or 5 wherein the cell culture medium contains human epidermal growth factor.

8. (Previously presented) The process of claim 1 wherein the cell culture medium comprises about 0.5-10 vol-% fetal bovine serum.

9. (Previously presented) The process of claim 1 further comprising trypsinizing the cultured cells at about 10^3-fold expansion, collecting the trypsinized cells by centrifugation, resuspending the collected cells in cell culture medium, and subjecting the resuspended cells to culture in contact with a fibronectin- and gelatin-coated surface.

10. (Original) The process of claim 1 wherein the cultured cells are subjected to cryopreservation.

11. (Original) The process of claim 10 wherein the cells are frozen in a cryopreservation medium comprising fetal calf serum containing an effective amount of dimethylsulfoxide.

12. (Original) The process of claim 10 or 11 wherein the cryopreserved cells are thawed and culturing is resumed in said cell culture medium.

13. (Original) The process of claim 1 wherein the expanded population comprises microvascular endothelial cells.

14. (Original) The process of claim 13 wherein the microvascular endothelial cells are $CD34^+$, $CD36^+$ and express the P1H1 antigen.

15-44. (Canceled)

45. (Previously presented) The process of claim 1 wherein the cell culture medium comprises hydrocortisone.

46. (Previously presented) The process of claim 1 wherein the cell culture medium comprises human serum.

47. (New) The process of claim 1 which does not employ antibodies to obtain buffy coat cells.

Remarks

Reconsideration and withdrawal of the rejections of the claims, in view of the remarks herein, is respectfully requested. Claim 47 is added. Claims 1-14 and 45-47 are pending.

Claim 47 is supported at page 3, lines 18-19 of the specification.

Substitute drawing sheets are enclosed herewith, addressing the objections noted on PTO-948 and the Examiner's comments at page 2 of the Office Action. In particular, drawing sheet 11 (Figure 5) is amended to indicate panels A and B, drawing sheet 13 is omitted as it is a substantial duplicate of drawing sheet 12 (Figure 6), and drawing sheet 14 (Figure 7) is amended so as to comply with 37 C.F.R. § 1.84(i).

The specification is amended at page 1, line 2 to indicate Applicant's claim to the benefit of the filing date of earlier-filed applications. A Petition under 37 C.F.R. § 1.78(a)(3) and § 1.78(a)(5) is enclosed herewith.

The Brief Description of Figure 5 is amended to refer to panels A and B.

The Examiner rejected claims 1-2, 5-6 and 8 under 35 U.S.C. § 103(a) as being unpatentable over Dzau et al. (U.S. Patent No. 6,352,555) in view of Asahara et al. (Science, 275:964 (1997)). The Examiner also rejected claims 1 and 3 under 35 U.S.C. § 103(a) as being unpatentable over Dzau et al. in view of Asahara et al. and further in view of Levine et al. (U.S. Patent No. 5,132,223). The Examiner also rejected claims 1, 4-5, 7, and 45-46 under 35 U.S.C. § 103(a) as being unpatentable over Dzau et al. in view of Asahara et al. and further in view of Gupta et al. (Exp. Cell Res., 230:244 (1997)). In addition, the Examiner rejected claims 1 and 13-14 under 35 U.S.C. § 103(a) as being unpatentable over Dzau et al. in view of Asahara et al. and further in view of Solovey et al. (NEJM, 337:1584 (1997)). Finally, the Examiner rejected claims 1 and 10-12 under 35 U.S.C. § 103(a) as being unpatentable over Dzau et al. in view of Asahara et al. and further in view of Dementriou et al. (U.S. Patent No. 6,140,123). These rejections are respectfully traversed.

The present application claims the benefit of the filing date of U.S. application Serial No. 60/109,687, filed on November 24, 1998. The Dzau et al. patent issued from U.S. application Serial No. 09/349,344, filed July 8, 1999, which claims the benefit of the filing date of U.S. application Serial No. 60/092,358, filed on July 10, 1998.

The Dzau et al. patent generally relates that a prosthesis composed of a porous tube, a portion of which has a certain diameter, can be contacted with cells and then exposed to a pressure differential to retain cells in the pores on the inner surface of the prosthesis (abstract). It is also disclosed that endothelial cells may be obtained by culturing a sample of mononuclear cells obtained from blood, e.g., the buffy coat, without further cell separation on a cell adhesive polymer-coated solid support, e.g., a fibronectin- or collagen-coated tissue culture plate, in the presence of endothelial growth factors, e.g., VEGF, bFGF and IGF, so as to result in an endothelial cell culture having at least 90% endothelial cells or progenitors thereof (column 3, lines 6-22, column 4, lines 32-36 and column 7, lines 13-61).

Nevertheless the Dzau et al. '358 application does not disclose or suggest a method for obtaining endothelial cells from blood, i.e., the sections corresponding to column 3, lines 6-22, column 4, lines 25-36, and column 7, lines 13-61 in the Dzau et al. patent are not present in the '358 application. Therefore, the disclosure in the Dzau et al. patent relating to obtaining endothelial cells from blood has an effective date of July 8, 1999 and so is not prior art to the present claims.

Asahara et al. relate the isolation of "putative endothelial cell (EC) progenitors or angioblasts from human peripheral blood" (abstract). It is disclosed that the cells were isolated by contacting human peripheral blood with magnetic beads coated with anti-CD34 or anti-Flk-1 antibodies (page 464). The resulting isolated cells ("MB^{CD34+} cells") were plated on tissue culture plastic or collagen, where "a limited number" of attached cells ("AT^{CD34+} cells") became spindle shaped, and proliferated for 4 weeks (page 964). It is also disclosed that a subset of MB^{CD34+} cells plated on fibronectin attached "promptly" and became spindle shaped within 3 days (page 904). The medium employed for cell culture experiments is described as M-199 with 20% FBS and bovine brain extract (footnote 14).

It is also disclosed that FACS analysis of AT^{CD34+} cells that were cultured on fibronectin for 7 days showed that those cells expressed CD34, CD31, Flk-1, Tie-2 and E selectin, all "markers of the EC lineage" (page 965). Asahara et al. relate that the injection of Dil-labeled MB^{CD34+} cells into the tail vein of mice with unilateral hind limb ischemia resulted in the integration of labeled cells in the neovascularized ischemic hind limb (page 965).

As disclosed in the specification, the cells in the expanded endothelial cell population have the typical "cobblestone" morphology of endothelial cells (page 6, lines 26-27 and page 18, lines 3-5). In contrast, the cells obtained by Asahara et al. were "spindle shaped". Therefore, Asahara et al. do not disclose or suggest a method to expand endothelial cells.

Levine et al. describe a method of culturing endothelial cells from solid tissue, i.e., from human blood vessels, such as human umbilical vein endothelial cells. The method employs a gelatin matrix supplemented with endothelial cell growth factor and heparin and/or a dextran sulfate (column 2, lines 3-14 and column 3, line 64-column 4, line 27). The resulting cultured cells were characterized as endothelial according to morphological and functional criteria (expression of "Factor VIII-related antigen") and production of angiotension-converting enzyme (column 4, lines 53-57). Levine et al. do not teach or suggest a method which employs buffy coat cells.

Gupta et al. describe a method to culture human dermal microvascular endothelial cells (HDMEC) in which cells are isolated by panning with EN4, an anti-endothelial cell monoclonal antibody, then stimulated with VEGF, a method which is intended to reduce fibroblast contamination of the resulting isolated population (page 244). It is disclosed that HDMEC were obtained from newborn human foreskin and, after trypsinization, the cells were cultured for three days prior to exposure of the cells to EN4 coated tissue culture plates (page 245). It is further disclosed that the panned cells, which were trypsinized and then cultured in gel-coated flasks, grew in circular colonies and exhibited cobblestone morphology (page 247). Gupta et al. do not mention buffy coat cells as a source for endothelial cells.

Solovey et al. disclose that immunohistochemical examination of buffy coat smears with P1H12, a monoclonal antibody which specifically reacts with endothelial cells, was used to enumerate circulating endothelial cells in normal blood donors, patients with sickle cell anemia and patients with non-hemoglobin S anemia (abstract and page 1585). For qualitative studies, circulating endothelial cells were isolated by two different methods: one employed P1H12 and the other employed P1H12-coated beads (page 1585). It is also disclosed that to determine whether P1H12-positive cells from the blood of patients with sickle cell anemia "remain alive", a population of P1H12-positive cells was contacted with an intracellularly-retained fluorescent dye and then cultured with primary microvascular endothelial cells for up to 28 days (citing to Gupta

et al.) (page 1586). Solovey et al. do not teach or suggest that circulating endothelial cells expand in culture.

Dementriou et al. disclose a method for preconditioning and cryopresentation of cells harvested from a donor (abstract). Dementriou et al. do not disclose or mention endothelial cells or a method to expand such cells.

With respect to the rejection of claims 1-2, 5-6, and 8 over Dzau et al. and Asahara et al.; claims 1 and 3 over Dzau et al., Asahara et al., and Levine et al.; claims 1, 4-5, 7, and 45-46 over Dzau et al., Asahara et al. and Gupta et al.; claims 1 and 13-14 over Dzau et al., Asahara et al. and Solovey et al.; and claims 1 and 10-12 over Dzau et al., Asahara et al., and Dementriou et al., as discussed above, Dzau et al. is not available as prior art to the pending claims. Moreover, as none of Asahara et al., Levine et al., Gupta et al., Solovey et al., or Dementriou et al. disclose or suggest a method to expand endothelial cells from buffy coat cells, there is no combination of the available cited art which renders Applicant's invention obvious.

Therefore, withdrawal of the § 103(a) rejections is respectfully requested.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-359-3265) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743

Respectfully submitted,

ROBERT P. HEBBEL ET AL.,

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
612-359-3265

Date August 21, 2003 By _____
Janet E. Embretson
Reg. No. 39,665

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 21st day of August, 2003.

Dawn M. Poole _____ Dawn M. Poole

Name Signature